Objective: To evaluate the safety of drugs used in infertility treatment.

Design: Literature search using the keywords birth defect, congenital malformation, clomiphene, aromatase inhibitor, letrozole, gonadotropin, metformin, gonadotropin releasing hormone agonist and antagonist, progesterone, progestin, and estrogen. We conducted the search in Medline, EMBASE, and Cochrane Database of systematic reviews.

Result(s): The available data suggest that clomiphene treatment, especially after several cycles, might be associated with a slightly higher risk of neural tube defects and severe hypospadias in the offspring. Letrozole and metformin do not appear to be teratogenic. The existing data concerning gonadotropin preparations suggest that there is no evidence of teratogenicity, yet, information after 1991 is lacking. Micronized progesterone, which is widely used in in vitro fertilization treatment, does not appear to increase the risk of nongenital birth defects; however, there might be a possible weak association between other progestational agents and hypospadias.

Conclusion(s): Infertility per se is a risk factor for congenital anomalies. Repeated clomiphene treatment might be associated with a slightly higher risk of hypospadias and neural tube defect. However, the overall increased risk related to various fertility drugs is only 1% to 2%. (Fertil Steril 2008;89:1595–602. ©2008 by American Society for Reproductive Medicine.)

Key Words: Congenital malformation, birth defect, infertility, gonadotropin, clomiphene, aromatase inhibitors, letrozole, metformin, progesterone, progestin, estrogen
The possible risk of neural tube defect (NTD) in the offspring was raised by early case reports and uncontrolled studies (8). The results have been mixed (9). Greenland and Ackerman (10) performed a pooled analysis of 10 epidemiologic controlled studies evaluating the relationship between CC and NTD. The estimated ratio of NTD prevalence among CC-exposed versus unexposed pregnancies ranged from 0.55 to 5.73 among the studies, but the variation was compatible with random fluctuation. The estimated prevalence ratio was 1.08 (95% confidence interval [CI] 0.76–1.51).

Medveczky et al. (11) evaluated 1,202 cases of NTD and compared them to 38,151 population controls without any birth defect and 22,475 patient controls with other defects. They found an odds ratio (OR) of 2.1 (95% CI 1.0–4.5) for CC and NTD. Their data suggest that there might be a slight and probable indirect association between the use of CC and NTD. Recently, Wu et al. (12) found an OR of 11.7 (95% CI 2.0–44.8) for NTD with the use of CC within the window spanning 60 days before to 15 days after conception. The number of CC cycles in the 12 months before conception was higher in mothers with infants with NTDs than in control mothers (mean 5.7 vs. 2.6 cycles, P=.01). In another study, Jain et al. (13) failed to show increased congenital malformation in women who conceived after high dose of CC (150–250 mg/day) compared with lower doses (50–100 mg/day). It seems that there is a weak association between the use of CC and NTD in the offspring (Table 1).

The dual antiestrogenic and estrogenic activity of CC (14, 15) in both male and female offspring is demonstrated by Nagao et al. (16). They administered 2, 4, or 8 mg/kg CC orally to 4-day-old rats, and found that male rats treated with high doses of CC for 10 weeks showed atrophy of seminiferous tubules and reduction in the number of spermatozoa in the epididymis. Female rats treated with either low or high doses of CC showed hypertrophy of the myometrium and glandular epithelium. These results suggest that CC can function as long-acting estrogen.

The chemical structure of CC is similar to diethylstilbestrol (DES), a drug associated with vaginal adenosis and infertility among DES-exposed female (DES daughters). Male offspring of these DES daughters had an increased risk of hypospadias (17). This raised a concern that the use of CC may be associated with hypospadias as well. Subsequently, Sørensen et al. (18) compared 319 cases of hypospadias with matched control male births without hypospadias. They found an adjusted odds ratio of 0.48 (95% CI 0.15–1.54) for hypospadias associated with CC, and concluded that CC is not associated with increased risk of hypospadias. This report, however, did not evaluate separately the different forms of hypospadias.

Meijer et al. (19) studying 392 cases of hypospadias from the European Registration of Congenital Anomalies and Twins reported otherwise. They found that the odds ratio for hypospadias in relation to preconceptional exposure to CC was 1.27 (95% CI 0.58–2.79). In contrast, for the OR for a severe form of hypospadias, the penoscrotal hypospadias was significantly higher (6.08, CI 95% 1.4–26.33) (Table 2). Data regarding the preconceptional exposure period to CC was available only from some patients, and it did not go beyond 3 months before the index pregnancy.

These results suggest that CC treatment, especially after several cycles, might be associated with a slightly higher risk of NTD and severe hypospadias in the offspring.

### AROMATASE INHIBITORS

Aromatase inhibitors are adjuvants for the treatment of breast cancer (20). Recently, several investigators used them for ovulation-inducing agents or superovulation (21–24). The most commonly used drugs for this purpose are nonsteroidal third-generation aromatase inhibitors, letrozole, and anastrazole. These compounds are absorbed completely after oral administration, with a mean half-life of approximately 45 hours (range 30–60 hours) (21). Mitwally et al. (21) first described the use of aromatase inhibitors for ovulation induction in women with polycystic ovary syndrome (PCOS) (22). Since then, several investigators reported the use of letrozole and anastrazole for different infertility treatments (21).

The potential teratogenic effect of letrozole were raised in an abstract presentation in 2005 (23); however, the study contains many flaws. Of a total 170 infants, 20 were lost to follow-up. As a result, 150 babies from 130 pregnancies were compared with a control group of over 36,000 infants born from low-risk pregnant women in a community hospital. The control population was younger than the letrozole group. They reported that the incidence of cardiac and “bone” anomalies were higher in the letrozole group than in the control group. The cardiac anomalies were comprised of two

<table>
<thead>
<tr>
<th>Investigators</th>
<th>Number</th>
<th>Odds ratio</th>
<th>95% Confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Greenland et al. (1995)</td>
<td>51 cases from 10 studies</td>
<td>1.08</td>
<td>0.76–1.51</td>
</tr>
<tr>
<td>Medveczky et al. (2004)</td>
<td>1,202 cases</td>
<td>2.1</td>
<td>1.0–4.5</td>
</tr>
<tr>
<td>Wu et al. (2006)</td>
<td>18 cases</td>
<td>11.7</td>
<td>2.0–44.8</td>
</tr>
</tbody>
</table>
cases of aortic stenosis in 150 babies, which the investigators calculated to be statistically higher than the rate of cardiac anomalies in the 36,000 babies born from low-risk pregnancies. Similarly, there were three different bone abnormalities in the letrozole babies, resulting in an apparent relative risk. These differences are solely related to type I error. In addition, the letrozole group consisted of babies born after letrozole, and combined letrozole and gonadotropins treatment, making it impossible to isolate the impact of letrozole alone.

Subsequently, Tulandi et al. (24) reported the outcome of newborns from mothers who conceived after treatment with letrozole (n = 514) and CC (n = 397). Overall, the congenital malformations and chromosomal abnormalities were not significantly different between the two groups (2.4% in the letrozole group compared with 4.8% in the CC group). The rate of major malformation in the letrozole group was 1.2% (6 of 514) and in the CC group was 3.0% (12 of 397). The miscarriage rate of letrozole-induced pregnancy is similar to that of CC (25).

In another study, Forman et al. (26), compared 112 newborns following letrozole treatment to 271 newborns following CC treatment and 94 newborns following spontaneous pregnancy. The rate of malformations was 0, 2.6%, and 3.2%, respectively. Therefore, the concern that letrozole use for ovulation induction could be teratogenic may be unfounded based on these data.

**METFORMIN**

Polycystic ovary syndrome affects 5% to 10% of women of reproductive age (27), and over 70% of women suffering from normogonadotropic anovulation present with ultrasound or endocrine features associated with PCOS (28). Because of the relationship between PCOS and insulin resistance, many of these women are treated with metformin.

Metformin (dimethylbiguanide) is an oral glucose-lowering agent to treat noninsulin dependent diabetes mellitus (NIDDM) (29). It acts mainly by improving the sensitivity of peripheral tissue (skeletal muscle) and the liver to insulin, thus opposing insulin resistance (30). In addition, it decreases basal hepatic glucose output, lowering fasting plasma glucose concentrations (31). Metformin was first suggested as a treatment for ovulation induction in women with PCOS in the early 1990s (32). Since then, it has been used increasingly in the treatment of infertility secondary to PCOS (33).

Several observational studies have also shown that metformin decreases the incidence of miscarriage in PCOS patients. Miscarriage occurred in 62% to 73% of pregnancies without metformin and 9% to 36% of pregnancies in the same women when metformin was taken (34–37). A recent study showed that compared with clomiphene treatment, there is no benefit of metformin on the incidence miscarriage in women with PCOS (38). However, metformin was discontinued when pregnancy test was positive.

Traditionally, treatment of NIDDM in pregnancy with the use of oral hypoglycemic drugs was contraindicated. This is because of the possible risks of fetal congenital anomaly (39–41). Recently, Gilbert et al. (42) reported the result of meta-analysis of eight studies that evaluated metformin treatment during pregnancy. All studies in their analysis had a control group, and all women were exposed in their first trimester to metformin. The pooled odds ratio, which was calculated only for major malformations, was 0.50 (95% CI 0.15–1.60) (Table 3). The investigators concluded that first-trimester exposure to metformin is not associated with an increased risk of major malformations. The Federal Drug Administration (FDA) classifies metformin as a category B drug. Data regarding the use of metformin throughout pregnancy or in cases of gestational diabetes mellitus and NIDDM pregnant women are still limited.

**GONADOTROPINS**

In 1958, Gemzel et al. (43) introduced the use of gonadotropin extract from human pituitaries to induce follicular growth in amenorrheic women. They reported 294 pregnancies resulting from human pituitary gonadotropins (hPG)-hCG treatment (44). Its use was limited by the shortage of human pituitaries. Subsequently, human urinary menopausal gonadotropins (hMG, menotropins) replaced hPG (45). Preliminary results on 66 infants born from hMG–hCG-treated mothers revealed 1 of 66 infants with major malformation and 5 of 66 with minor malformation (46). In a review of 1,160 babies born following ovulation induction with hMG–hCG (47), the investigators found 63 infants with malformations. These
represent an overall incidence of 54.3 per 1,000 (major malformation 21.6 of 1,000, minor malformations 32.7 of 1,000). The rates are similar to those in the general population (Table 3). Therefore, the existing data suggest that there is no evidence of teratogenicity of gonadotropin preparations.

The gonadotropins used in the previous reports were prepared from a human urinary source and were prescribed using similar dosages to those used today for ovulation induction. Today, many physicians use recombinant gonadotropin preparation in very high doses, especially for in vitro fertilization (IVF) treatment. Because of the paucity of information after 1991, the time has come to reevaluate the risk of congenital malformation in the offspring of gonadotropin-treated mothers.

PROGESTERONE

Progesterone was one of the first steroidal hormones studied as a contraception in the 1950s (48). Later, it was used to support high-risk pregnancies, including repeated miscarriages (49). Commercially marketed “natural” progesterone (which is the exact chemical duplicate of the hormone) is synthesized using the plant steroid diosgenin as a precursor. Because of its low oral bioavailability, it was soon replaced by other synthetic progestational agents (with a modified chemical structure) that have greater oral bioavailability. These agents are classified as “progestins,” “progestagens,” or “gestagens” based on their similarity to progesterone in the Clauberg assay (50), an early bioassay that measured response to the estrogen-primed rabbit endometrium. In contrast to “natural” progesterone, these agents have greater oral bioavailability, and their effects on different endocrine organs differ. Based on the structural similarity with the respective receptors, they may act as weak androgens or antiandrogens, glucocorticoids, or antimineralocorticoids (51).

In the past several years, the “natural” progesterone has returned in a “micronized” form with enhanced bioavailability. It was used first in Europe (52), and in 1998, the FDA approved its use in the Unites States. Currently, it is widely used for luteal support in IVF treatment (53).

It is reassuring that studies in rats and monkeys have not shown an increased rate of progesterone-related congenital anomalies (54). More importantly, a collaborative study from West Germany, which included 186 progesterone-exposed pregnancies, could not find an increase in birth defects with progesterone (55). Three clinical studies support their findings (56–58). Yet, Rock et al. (57) reported 2 congenital malformations (undescended unilateral testis and meningomyelocele) among 93 women treated with progesterone during the first trimester of pregnancy. Check et al. (58) found five congenital malformations (two cardiovascular, omphalocele, hydrocephaly, and club foot with cleft palate) in 382 women exposed to either progesterone or 17α-hydroxyprogesterone. These studies had no control group.

In a case–control study, the investigators reported a relationship between the use of hormones including progesterone and isolated hypospadias (59, 60). Because of the lack of association between the timing of hormone therapy and the location of the urethral orifice, and of the severity of the disorder and hormone therapy, the investigators concluded that their findings were more likely to be associated with recall or interviewer bias. Using data from the National Birth Defects Prevention Study, Carmichael et al. (61), studied the risk of second- or third-degree hypospadias in association with periconceptional progestin intake. Progestin-related hypospadias was reported by 42 (8.4%) case mothers and 31 (2.4%) control mothers, for intakes from 4 weeks before conception to 14 weeks after. The crude odds ratio for progestin use at any time was 3.7 (95% CI 2.3–6.0) (Table 3). However, information of the dose and method of administration for most women was not available. In addition, the type of progestin and indication were not specified in detail. Data on maternal and paternal fertility were also limited.

In 1999, the FDA revoked pregnancy warning labels for progestational agents (62), and they classified micronized progesterone, which is widely used in IVF treatments, as a category B drug. It appears that progesterone treatment does not increase the risk of nongenital birth defects. Although a few investigators reported an association between

<table>
<thead>
<tr>
<th>Type of fertility drug</th>
<th>Risk of congenital malformation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clomiphene citrate</td>
<td>Slightly higher risk of neural tube defect and severe hypospadias especially after multiple cycles</td>
</tr>
<tr>
<td>Letrozole</td>
<td>No known increased risk</td>
</tr>
<tr>
<td>Metformin</td>
<td>No known increased risk</td>
</tr>
<tr>
<td>Gonadotropin</td>
<td>No known increased risk, but no study after 1991</td>
</tr>
<tr>
<td>Progesterone</td>
<td>Might increase risk of hypospadias.</td>
</tr>
<tr>
<td>Estrogen</td>
<td>No known increased risk</td>
</tr>
<tr>
<td>GnRH agonist</td>
<td>No known increased risk</td>
</tr>
<tr>
<td>GnRH antagonist</td>
<td>Limited data</td>
</tr>
</tbody>
</table>

progesterone treatment and hypospadias, its relationship with the widely used “natural” micronized progesterone is unlikely. In a study of inadvertent use of depot medroxyprogesterone acetate during pregnancy, no fetal malformation was reported (63).

ESTROGENS

The first orally active estrogen synthesized in 1938 was DES. It has structural similarities to 17β estradiol. It was used for prevention of spontaneous abortions and preterm deliveries. Later, it was found to be associated with various gross anomalies of the genital tract and clear cell adenocarcinoma of the vagina and uterine cervix in daughters exposed in utero to DES. This led to concerns using estrogen preparation in early pregnancy.

Some investigators advocated the use of estrogens in addition to progesterone for luteal support in IVF treatment. Among these are conjugated equine estrogens, ethinyl estradiol, estrone, and estradiol. Early mouse studies demonstrated teratogenicity of estradiol salts, including cleft palate with estradiol benzoate (64) and eyelid and mammary defects with estradiol dipropionate (65). However, studies in monkeys using estradiol salts in combination with hydroxyprogesterone caproate failed to show a relationship between estrogen and congenital anomalies (66).

Epidemiologic studies in humans are limited by the use of other substances in combination with estrogen, and by the failure to distinguish different types of estrogens and progestins. Nevertheless, at least three studies demonstrated that estradiol use during pregnancy does not increase the risk of congenital anomalies (55, 67, 68). In addition, two meta-analyses could not find any association between first-trimester exposure to oral contraceptives and malformations including external genital defects (69, 70).

GONADOTROPIN RELEASING HORMONE AGONISTS (GnRHa)

GnRH agonists are widely used in IVF treatment as ovulation suppressing agents, and the most commonly used are buserelin and leuprolide acetate. Compared with native GnRH that has a half-life of 2 to 4 minutes, their plasma half-life is a few times longer. Studies in animal models demonstrated that when pregnant rabbits were exposed to buserelin during the period of organogenesis in doses similar to or lower than those used in humans, their offspring had increased rates of fetal death and skeletal anomalies (71). Most likely, this is species-specific, as the same effects were not seen in mice (72).

To date, there have been 255 infants born to women who were inadvertently treated with buserelin in pregnancy. Four newborns (1.6%) had congenital anomalies, but no consistent type of anomaly was found (73). Similarly, there have been over 125 cases of early human pregnancies exposed to leuprolide acetate (74). These include 35 pregnancies (28%) that miscarried, a second-trimester pregnancy loss because of an incompetent cervix, one pregnancy termination for trisomy 18, and three ectopic pregnancies. Of two infants with congenital anomalies, one had a bilateral inguinal hernia and another had an atretic kidney (75). In another series of 28 pregnancies exposed to triptoreline acetate, there were no abnormalities (76).

In 1999, Lahat et al. (77) evaluated six children who were exposed to GnRHa around the time of conception. The types of GnRHa were not specified. They reported that a child had a cleft palate and four others had neurologic problems including epilepsy in one child and attention-deficit/hyperactivity disorder in the remainder. In a group of 20 control children, there was only one with a diagnosis of attention-deficit/hyperactivity disorder. The investigators appealed for a long-term neurologic evaluation of children exposed to GnRHa.

Based on basic and clinical studies, it appears that GnRHa use in infertile women is safe. In any event, one should be vigilant of not administrating GnRHa to pregnant women.

GONADOTROPIN RELEASING HORMONE ANTAGONISTS

Similar to GnRH agonists, the antagonists such as cetrorelix and ganirelix are used to suppress ovulation during IVF treatments. Their half-life does not exceed 60 hours, and similar to GnRH agonists, GnRH antagonists are not expected to be present during the time of implantation. To the best of our knowledge, there has been no report of inadvertent use of GnRHa antagonist in pregnancy. However, there are three reports of congenital malformations following IVF cycles with GnRHa antagonists (78–80). The cause–effect relationship is unclear.

Ludwig et al. (78) reported a major malformation rate of 3.1% (7 of 232) and minor malformations rate of 2.6% (6 of 232) among babies born from mothers treated with IVF using the antagonist and gonadotropins. Olivennes et al. (79) followed 67 pregnant women following IVF treatments using ganirelix. Five newborns had minor congenital malformations (6.8%), and one had a major congenital malformation (1.4%). In another study, Boerrigter et al. (80) reported the outcome of 424 children born following IVF cycles using ganirelix. The congenital malformation rate was 7.5% (32 of 424 children), similar to that observed in children born following GnRHa agonist IVF cycles (5.5%, 10 of 181 children).

Although the data is limited, it seems that GnRHa antagonists are not associated with increased risk of congenital malformation.

INFERTILITY AS A RISK FACTOR FOR CONGENITAL MALFORMATION

In most studies evaluating the risk of congenital malformation following infertility treatment, the major confounding factor is the association of infertility itself with congenital malformation. Zhu et al. (81) compared the prevalence of congenital malformation of infants born to fertile couples,
infertile couples who conceived spontaneously, and infertile couples who conceived following infertility treatment. Compared with singletons born from fertile mothers, singletons born from infertile mothers who conceived spontaneously or after treatment had a higher prevalence of congenital malformation (hazard ratio [HR] 1.20, 95% CI 1.07–1.35 and 1.39, 95% CI 1.23–1.57, respectively). They also evaluated different infertility treatments including intracytoplasmic sperm injection (ICSI), IVF, intrauterine insemination (IUI), hormonal treatment (without IUI), and surgery. The HR for congenital malformation in offspring of the IUI group compared with those of fertile couple was 1.33 (95% CI 1.07–1.65). For genital organ malformation, the HR was higher (2.07, 95% CI 1.05–4.06). In the IVF group the HR was 2.24 (95% CI 1.17–4.28), and in the ICSI group 3.93 (95% CI 1.61–9.61). It appears that as the male factor becomes more severe, the risks of genital organ malformation increase.

In the hormonal treatment group, the rate of genital organ malformation was the same as in the fertile group. However, compared with the fertile couple group, the HR for nervous system malformation in the hormonal treatment group was 2.79 (95% CI 1.13–6.92).

CONCLUSIONS
Infertility treatments bypass different barriers that normally prevent the infertile couple from conceiving. Consequently, women deliver babies that perhaps would have not been born otherwise. These offspring are at risk for the development of congenital anomaly. However, compared with fertile couples, the overall increased risk following infertility treatment is only 1% to 2%. It should be noted that infertility per se is probably an independent risk factor for congenital malformations.

For non-IVF treatment, the available data suggest that CC treatment, especially after several cycles, might be associated with a slightly higher risk of NTD and severe hypospasias in the offspring. Aromatase inhibitors could replace CC, as recent reports suggest that letrozole use for ovulation induction may not be associated with increased risk of fetal anomaly.

For IVF treatment, physicians administer not only a wide variety of drugs, but also in high doses. It is reassuring that most medications used for IVF appear to be safe. There is no or minimal risk of congenital malformation associated with GnRH agonist and antagonist, gonadotropin, estrogen, or progesterone. Nevertheless, most studies evaluating the possible relationship between birth defect and gonadotropin were in the 1980s and early 1990s. We are now using enormous doses of gonadotropin, and it is time to reevaluate the safety of gonadotropin treatment to the offspring. The DES experience is a lesson for us to be vigilant.

REFERENCES
3. Adashi EY. Clomiphene citrate: mechanism(s) and site(s) of action—a hypothesis revisited. Fertil Steril 1984;42:331–44.


